Nuclear magnetic resonance-based metabolomics identifies phenylalanine as a novel predictor of incident heart failure hospitalisation : results from PROSPER and FINRISK 1997

Delles, Christian

2018-04

Delles , C , Rankin , N J , Boachie , C , McConnachie , A , Ford , I , Kangas , A , Soininen , P , Trompet , S , Mooijaart , S P , Jukema , J W , Zannad , F , Ala-Korpela , M , Salomaa , V , Havulinna , A S , Welsh , P , Wurtz , P & Sattar , N 2018 , ' Nuclear magnetic resonance-based metabolomics identifies phenylalanine as a novel predictor of incident heart failure hospitalisation : results from PROSPER and FINRISK 1997 ' , European Journal of Heart Failure , vol. 20 , no. 4 , pp. 663-673 . https://doi.org/10.1002/ejhf.1076

http://hdl.handle.net/10138/237142
https://doi.org/10.1002/ejhf.1076

unspecified
publishedVersion

European Journal of Heart Failure (2018) **20**, 663–673
doi:10.1002/ejhf.1076

RESEARCH ARTICLE

Nuclear magnetic resonance-based metabolomics identifies phenylalanine as a novel predictor of incident heart failure hospitalisation: results from PROSPER and FINRISK 1997

Christian Delles[1†], Naomi J. Rankin[1,2*†], Charles Boachie[3], Alex McConnachie[3], Ian Ford[3], Antti Kangas[4], Pasi Soininen[4,5], Stella Trompet[6], Simon P. Mooijaart[6], J. Wouter Jukema[6], Faiez Zannad[7,8], Mika Ala-Korpela[4,5,9,10,11,12], Veikko Salomaa[13], Aki S. Havulinna[13,14], Paul Welsh[1], Peter Würtz[15], and Naveed Sattar[1]

[1]Institute of Cardiovascular and Medical Sciences (ICAMS), BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK; [2]Glasgow Polyomics, Joseph Black Building, University of Glasgow, Glasgow, UK; [3]Robertson Centre for Biostatistics, Boyd Orr Building, University of Glasgow, Glasgow, UK; [4]Computational Medicine, Faculty of Medicine and Biocenter Oulu, University of Oulu, Oulu, Finland; [5]NMR Metabolomics Laboratory, School of Pharmacy, University of Eastern Finland, Kuopio, Finland; [6]Leiden University Medical Centre, Leiden, The Netherlands; [7]Inserm Centre d'Investigation Clinique (CIC) 1443, Université de Lorraine, Lorraine, France; [8]Centre Hospitalier Régional Universitaire (CHRU) de Nancy, Nancy, France; [9]Medical Research Council Integrative Epidemiology Unit, University of Bristol, Bristol, UK; [10]Population Health Science, Bristol Medical School, University of Bristol, Bristol, UK; [11]Systems Epidemiology, Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia; [12]Department of Epidemiology and Preventive Medicine, School of Public Health and Preventive Medicine, Faculty of Medicine, Nursing and Health Sciences, The Alfred Hospital, Monash University, Melbourne, Victoria, Australia; [13]National Institute for Health and Welfare (THL), Helsinki, Finland; [14]Institute for Molecular Medicine (FIMM), University of Helsinki, Helsinki, Finland; and [15]Research Programs Unit, Diabetes and Obesity, University of Helsinki, Helsinki, Finland

Received 1 October 2017; revised 5 October 2017; accepted 11 October 2017; online publish-ahead-of-print 11 December 2017

Aims We investigated the association between quantified metabolite, lipid and lipoprotein measures and incident heart failure hospitalisation (HFH) in the elderly, and examined whether circulating metabolic measures improve HFH prediction.

Methods and results Overall, 80 metabolic measures from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) trial were measured by proton nuclear magnetic resonance spectroscopy ($n = 5341$; 182 HFH events during 2.7-year follow-up). We repeated the work in FINRISK 1997 ($n = 7330$; 133 HFH events during 5-year follow-up). In PROSPER, the circulating concentrations of 13 metabolic measures were found to be significantly different in those who were later hospitalised for heart failure after correction for multiple comparisons. These included creatinine, phenylalanine, glycoprotein acetyls, 3-hydroxybutyrate, and various high-density lipoprotein measures. In Cox models, two metabolites were associated with risk of HFH after adjustment for clinical risk factors and N-terminal pro-B-type natriuretic peptide (NT-proBNP): phenylalanine [hazard ratio (HR) 1.29, 95% confidence interval (CI) 1.10–1.53; $P = 0.002$] and acetate (HR 0.81, 95% CI 0.68–0.98; $P = 0.026$). Both were retained in the final model after backward elimination. Compared to a model with established risk factors and NT-proBNP, this model did not improve the C-index but did improve the overall continuous net reclassification index (NRI 0.21; 95% CI 0.06–0.35; $P = 0.007$) due to improvement in classification of non-cases (NRI 0.14; 95% CI 0.12–0.17; $P < 0.001$). Phenylalanine was replicated as a predictor of HFH in FINRISK 1997 (HR 1.23, 95% CI 1.03–1.48; $P = 0.023$).

Conclusion Our findings identify phenylalanine as a novel predictor of incident HFH, although prediction gains are low. Further mechanistic studies appear warranted.

Keywords Metabolomics • Advanced lipoprotein profiling • Heart failure • PROSPER • Phenylalanine • FINRISK

*Corresponding author. B4.18a Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, UK. Tel: +44 0141 300 3364, Email: naomi.rankin@glasgow.ac.uk
†These authors should be regarded as joint first authors.

Introduction

The prevention of heart failure (HF) is an important clinical issue. Patients with HF have a high mortality and impaired quality of life, so identifying those at risk is important.[1,2] The risk of HF increases with age but typical symptoms of HF, such as shortness of breath, may be absent in the elderly (or masked by other co-morbidities); the prognosis of HF is poor, and the mechanisms of HF differ in the elderly.[3] Treatment of hypertension and dyslipidaemia, prevention of diabetes, smoking cessation, increased exercise, weight reduction, and reduced alcohol intake have been associated with lower risks for HF.[4–6] At present, symptomatic patients have B-type natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP) concentrations measured as a rule-out test for HF, a cost-effective strategy to increase the positive predictive value of echocardiography. However, routine screening for this marker as part of cardiovascular disease (CVD) risk screening is not cost-effective as the assay is currently much more expensive than other routine clinical laboratory tests.[7] More effective screening for prevalent HF, perhaps using 'omics technologies, in combination with more effective interventions, has been described as an urgent need in the HF clinical arena.[7] Such strategies might help pave the way toward better identification of HF, or identify novel treatment strategies. Studies to improve the understanding of HF aetiology and generate new hypothesis, particularly in the elderly, are also needed.

Metabolomics is the study of the small molecule complement of a system using a variety of methods, mainly mass spectrometry (MS) and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy.[8] Both methods are complementary, each with their own strengths and limitations.[8] MS metabolomics methods are generally very sensitive, detecting thousands of metabolites, but routinely provide only relative (rather than absolute) quantitation. Generally, ^{1}H-NMR metabolomics methods have poor sensitivity in comparison to MS but do provide absolute quantitation, higher throughput (resulting in reduced costs), and better reproducibility. Metabolomics is of particular interest, since HF is strongly linked to metabolic dysfunction. Dysregulation of cardiac energy metabolism and cardiac remodelling are key features of HF that may result in changes in circulating metabolite concentrations, and adverse metabolic states like diabetes increase HF risk.[9–11] Whether changes in metabolic profile precede incident HF is therefore an important mechanistic line of research. Metabolomics has been used to study prevalent HF,[9,10,12–20] but most studies have been cross-sectional in nature. Presently, only one study has prospectively investigated the association of the metabolome with future HF risk.[13] This study used an untargeted gas chromatography/MS metabolomics method to identify two metabolites associated with incident HF.[13] In contrast, we here employ a ^{1}H-NMR spectroscopy method that allowed detailed lipoprotein subclass analysis, in addition to small molecule and lipid concentrations,[21] to study samples from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) trial.[22] We hypothesised that metabolites, lipids and lipoproteins would associate with HF hospitalisation (HFH) in elderly men and women and improve HFH prediction beyond established clinical predictors and NT-proBNP.

Methods

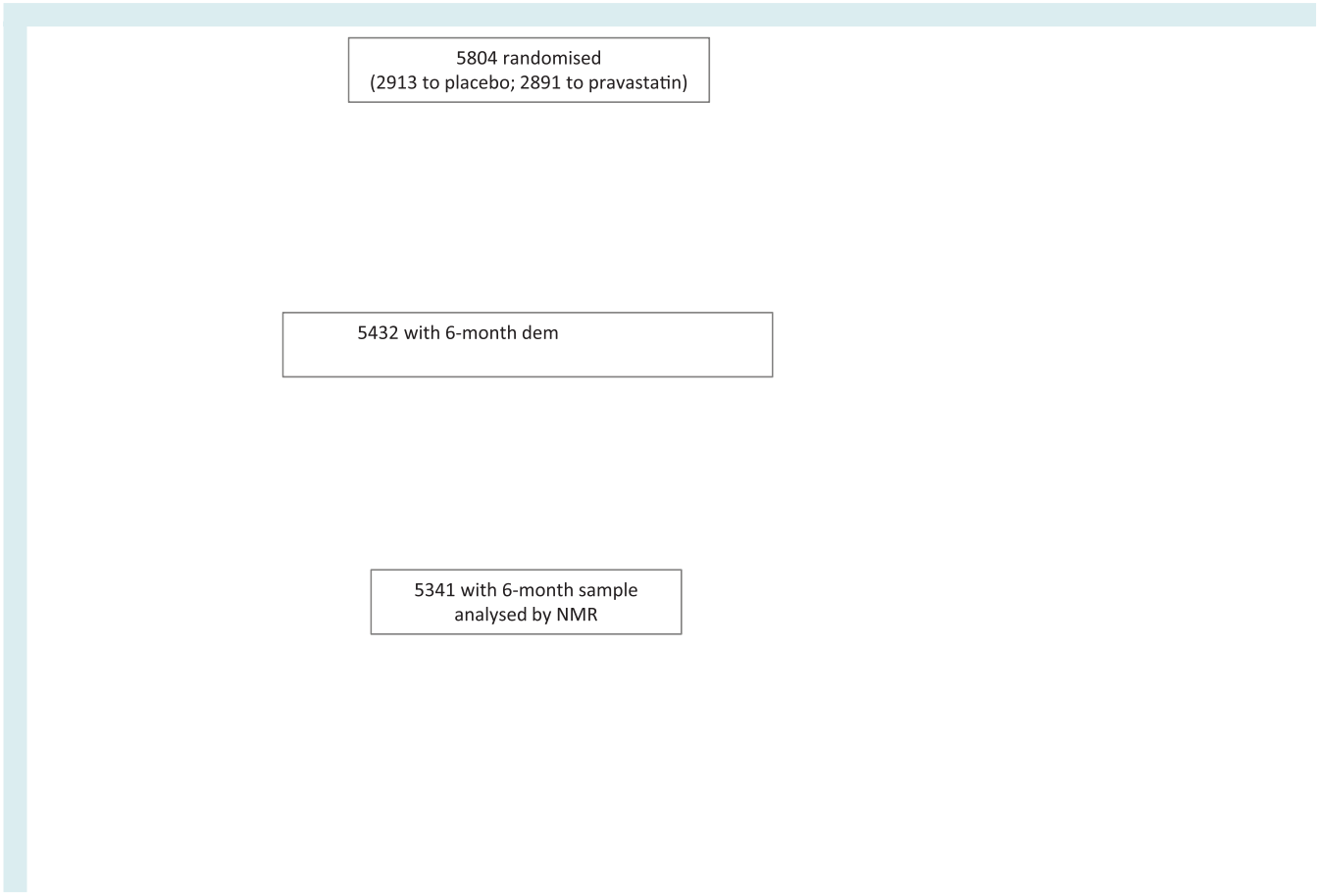
Study cohort: PROSPER

The PROSPER trial design has been published.[22] In brief, this was a double-blind, randomised, placebo-controlled trial investigating the benefit of pravastatin (40 mg/day) in elderly individuals at risk of CVD. Participants were identified in the primary care setting from three centres: Glasgow, Scotland; Cork, Ireland; and Leiden, The Netherlands. Overall, 5804 elderly adults (70–82 years old) were enrolled. All participants had high-normal to high cholesterol concentrations (4.0–9.0 mmol/L) at baseline. Additionally, 50% of patients had evidence of vascular disease (physician diagnosed stable angina, stroke, transient ischaemic attack, or myocardial infarction) and the remaining 50% of patients had high risk of vascular disease as they had either hypertension, diabetes, or were smokers. Individuals with congestive HF [New York Heart Association (NYHA) class III and IV] were excluded. The primary outcome measure of PROSPER was a composite CVD outcome. In the current study, the endpoint of interest was hospitalisation for incident HF. This was defined based on a combination of symptoms (e.g. shortness of breath) and signs, including chest radiograph with fluid congestion or echocardiogram with severely diminished left ventricular function.[23] Patients were recruited between December 1997 and May 1999, and the mean follow-up period was 3.2 years.[24] The investigation conforms with the principles outlined in the Declaration of Helsinki. The institutional ethics review boards of all three European centres approved the study protocol.[25] All participants provided written informed consent to participate in the study and for long-term follow-up.

Fasting venous blood samples were collected at baseline and at 3-month intervals and biobanked at −80 °C. For the present study, previously unthawed 6-month post-randomisation samples were used, employing the study as a cohort study and adjusting for randomised treatment in analysis. Overall, 5341 samples were available for this study, having sample available for ^{1}H-NMR analysis and available 6-month NT-proBNP and other measurements[24] (*Figure 1*). Estimated glomerular filtration rate (eGFR) was calculated based on routinely available creatinine, using the Modification of Diet in Renal Disease (MDRD) equation.[26] Eighteen participants who had died or experienced HFH in the first 6 months of follow-up were excluded from the analysis since 6-month samples were used as predictors of incident HFH.

External replication cohort: FINRISK

The population-based FINRISK 1997 cohort was used as an external replication cohort. Participants were aged between 25 and 74 years at recruitment and were derived from the general population in five study areas across Finland.[27] All participants provided written informed consent and the study protocol was approved by the local ethics committees. A total of 8444 individuals were recruited and NMR metabolic measures were available for 7602 baseline serum samples. Semi-fasting venous blood samples were biobanked at −80 °C. For the present study, samples with only one previous freeze–thaw cycle were used. Incident HF during follow-up was identified through the Finnish National Hospital Discharge Register and Cause-of-Death Register using the International Classification of Diseases diagnosis codes, 10th revision. Additionally, nationwide drug reimbursement and prescription registers were used to identify individuals on HF medication. This method of registry follow-up has been validated.[27] We curtailed follow-up to 5 years (longer than the 2.7 years in PROSPER)


5804 randomised
(2913 to placebo; 2891 to pravastatin)
5432 with 6-month dem
5341 with 6-month sample
analysed by NMR

the 7th Framework Programme (grant #305507) "Heart 'omics' in AGEing" (HOMAGE). This study was further supported by the Strategic Research Funding from the University of Oulu, Finland, the Sigrid Juselius Foundation, the Novo Nordisk Foundation, and the Academy of Finland (grant #294834). The serum NMR metabolomics platform has been supported by the Sigrid Juselius Foundation and the Strategic Research Funding from the University of Oulu. M.A.K. works in a Unit that is supported by the University of Bristol and UK Medical Research Council (MC_UU_1201/1). V.S. was supported by the Finnish Foundation for Cardiovascular Research. N.J.R. was supported by the Glasgow Molecular Pathology NODE, funded by The Medical Research Council and The Engineering and Physical Sciences Research Council (MR/N005813/1, project code 69042/1).

Conflicts of interest: N.S. has consulted for AstraZeneca, Bristol-Myers Squibb, Amgen, Sanofi and Boehringer Ingelheim. A.K., P.S., and P.W. have employment relation with Brainshake Ltd, and are shareholders of Brainshake Ltd, a company offering NMR-based metabolite and lipoprotein profiling. The other authors report no conflict of interest.

References

1. Bui AL, Horwich TB, Fonarow GC. Epidemiology and risk profile of heart failure. *Nat Rev Cardiol* 2011;**8**:30–41.
2. McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Böhm M, Dickstein K, Falk V, Filippatos G, Fonseca C, Gomez-Sanchez MA, Jaarsma T, Køber L, Lip GY, Maggioni AP, Parkhomenko A, Pieske BM, Popescu BA, Rønnevik PK, Rutten FH, Schwitter J, Seferovic P, Stepinska J, Trindade PT, Voors AA, Zannad F, Zeiher A. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2012;**14**:803–869.
3. Lazzarini V, Mentz RJ, Fiuzat M, Metra M, O'Connor CM. Heart failure in elderly patients: distinctive features and unresolved issues. *Eur J Heart Fail* 2013;**15**:717–723.
4. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, Jessup M, Konstam MA, Mancini DM, Michl K, Oates JA, Rahko PS, Silver MA, Stevenson LW, Yancy CW. 2009 Focused update incorporated into the ACC/AHA 2005 guidelines for the diagnosis and management of heart failure in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Developed in collaboration with the International Society for Heart and Lung Transplantation. *J Am Coll Cardiol* 2009;**53**:e1–e90.
5. Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldman AM, Francis GS, Ganiats TG, Goldstein S, Gregoratos G, Jessup ML, Noble RJ, Packer M, Silver MA, Stevenson LW, Gibbons RJ, Antman EM, Alpert JS, Faxon DP, Fuster V, Gregoratos G, Jacobs AK, Hiratzka LF, Russell RO, Smith SC Jr. ACC/AHA Guidelines for the evaluation and management of chronic heart failure in the adult: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1995 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the International Society for Heart and Lung Transplantation; endorsed by the Heart Failure Society of America. *Circulation* 2001;**104**:2996–3007.
6. Echouffo-Tcheugui JB, Greene SJ, Papadimitriou L, Zannad F, Yancy CW, Gheorghiade M, Butler J. Population risk prediction models for incident heart failure: a systematic review. *Circ Heart Fail* 2015;**8**:438–447.
7. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJ, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WH, Tsai EJ, Wilkoff BL. 2013 ACCF/AHA Guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2013;**62**:e147–e239.
8. Rankin NJ, Preiss D, Welsh P, Burgess KE, Nelson SM, Lawlor DA, Sattar N. The emergence of proton nuclear magnetic resonance metabolomics in the cardiovascular arena as viewed from a clinical perspective. *Atherosclerosis* 2014;**237**:287–300.
9. Lin D, Hollander Z, Meredith A, Stadnick E, Sasaki M, Cohen Freue G, Qasimi P, Mui A, Ng RT, Balshaw R, Wilson-McManus JE, Wishart D, Hau D, Keown PA, McMaster R, McManus BM; Biomarkers in Transplantation Team; NCE CECR PROOF Centre of Excellence. Molecular signatures of end-stage heart failure. *J Card Fail* 2011;**17**:867–874.
10. Dunn WB, Broadhurst DI, Deepak SM, Buch MH, McDowell G, Spasic I, Ellis DI, Brooks N, Kell DB, Neyses L. Serum metabolomics reveals many novel metabolic markers of heart failure, including pseudouridine and 2-oxoglutarate. *Metabolomics* 2007;**3**:413–426.
11. Welsh P, McMurray JJ. B-type natriuretic peptide and glycaemia: an emerging cardiometabolic pathway? *Diabetologia* 2012;**55**:1240–1243.
12. Cheng ML, Wang CH, Shiao MS, Liu MH, Huang YY, Huang CY, Mao CT, Lin JF, Ho HY, Yang NI. Metabolic disturbances identified in plasma are associated with outcomes in patients with heart failure: *diagnostic and prognostic value of metabolomics*. *J Am Coll Cardiol* 2015;**65**:1509–1520.
13. Zheng Y, Yu B, Alexander D, Manolio TA, Aguilar D, Coresh J, Heiss G, Boerwinkle E, Nettleton JA. Associations between metabolomic compounds and incident heart failure among African Americans: the ARIC Study. *Am J Epidemiol* 2013;**178**:534–542.
14. Wang J, Li Z, Chen J, Zhao H, Luo L, Chen C, Xu X, Zhang W, Gao K, Li B, Zhang J, Wang W. Metabolomic identification of diagnostic plasma biomarkers in humans with chronic heart failure. *Mol Biosyst* 2013;**9**:2618–2626.
15. Tenori L, Hu X, Pantaleo P, Alterini B, Castelli G, Olivotto I, Bertini I, Luchinat C, Gensini GF. Metabolomic fingerprint of heart failure in humans: a nuclear magnetic resonance spectroscopy analysis. *Int J Cardiol* 2013;**168**:e113–e115.
16. Luan H, Chen X, Zhong S, Yuan X, Meng N, Zhang J, Fu J, Xu R, Lee C, Song S, Jiang H, Xu X. Serum metabolomics reveals lipid metabolism variation between coronary artery disease and congestive heart failure: a pilot study. *Biomarkers* 2013;**18**:314–321.
17. Alexander D, Lombardi R, Rodriguez G, Mitchell MM, Marian AJ. Metabolomic distinction and insights into the pathogenesis of human primary dilated cardiomyopathy. *Eur J Clin Invest* 2011;**41**:527–538.
18. Turer AT. Using metabolomics to assess myocardial metabolism and energetics in heart failure. *J Mol Cell Cardiol* 2013;**55**:12–18.
19. Zordoky BN, Sung MM, Ezekowitz J, Mandal R, Han B, Bjorndahl TC, Bouatra S, Anderson T, Oudit GY, Wishart DS, Dyck JR; Alberta HEART. Metabolomic fingerprint of heart failure with preserved ejection fraction. *PLoS One* 2015;**10**:e0124844.
20. Bedi KC, Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, Wang LL, Javaheri A, Blair IA, Margulies KB, Rame JE. Evidence for intramyocardial disruption of lipid metabolism and increased myocardial ketone utilization in advanced human heart failure. *Circulation* 2016;**133**:706–716.
21. Soininen P, Kangas AJ, Wurtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet* 2015;**8**:192–206.
22. Shepherd J, Blauw GJ, Murphy MB, Cobbe SM, Bollen EL, Buckley BM, Ford I, Jukema JW, Hyland M, Gaw A, Lagaay AM, Perry IJ, Macfarlane PW, Meinders AE, Sweeney BJ, Packard CJ, Westendorp RG, Twomey C, Stott DJ. The design of a prospective study of Pravastatin in the Elderly at Risk (PROSPER). PROSPER Study Group. PROspective Study of Pravastatin in the Elderly at Risk. *Am J Cardiol* 1999;**84**:1192–1197.
23. Nanchen D, Stott DJ, Gussekloo J, Mooijaart SP, Westendorp RG, Jukema JW, Macfarlane PW, Cornuz J, Rodondi N, Buckley BM, Ford I, Sattar N, de Craen AJ; PROSPER Group. Resting heart rate and incident heart failure and cardiovascular mortality in older adults: role of inflammation and endothelial dysfunction: the PROSPER study. *Eur J Heart Fail* 2013;**15**:581–588.
24. Poortvliet RK, van Peet PG, de Craen AJ, Mertens BJ, Mooijaart SP, Wijsman LW, Drewes YM, Ford I, Sattar N, Jukema JW, Stott DJ, de Ruijter W, Gussekloo J. Risk stratification and treatment effect of statins in secondary cardiovascular prevention in old age: additive value of N-terminal pro-B-type natriuretic peptide. *Eur J Prev Cardiol* 2016;**23**:1104–1113.
25. Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM, Ford I, Gaw A, Hyland M, Jukema JW, Kamper AM, Macfarlane PW, Meinders AE, Norrie J, Packard CJ, Perry IJ, Stott DJ, Sweeney BJ, Twomey C, Westendorp RG; PROSPER study group. PROspective Study of Pravastatin in the Elderly at Risk. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. *Lancet* 2002;**360**:1623–1630.
26. Ford I, Bezlyak V, Stott DJ, Sattar N, Packard CJ, Perry I, Buckley BM, Jukema JW, de Craen AJ, Westendorp RG, Shepherd J. Reduced glomerular filtration rate and its association with clinical outcome in older patients at risk of vascular events: secondary analysis. *PLoS Med* 2009;**6**:e16.

27. Wurtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, Ghorbani A, Artati A, Wang Q, Tiainen M, Kangas AJ, Kettunen J, Kaikkonen J, Mikkilä V, Jula A, Kähönen M, Lehtimäki T, Lawlor DA, Gaunt TR, Hughes AD, Sattar N, Illig T, Adamski J, Wang TJ, Perola M, Ripatti S, Vasan RS, Raitakari OT, Gerszten RE, Casas JP, Chaturvedi N, Ala-Korpela M, Salomaa V. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation* 2015;**131**:774–785.
28. Inouye M, Kettunen J, Soininen P, Silander K, Ripatti S, Kumpula LS, Hämäläinen E, Jousilahti P, Kangas AJ, Männistö S, Savolainen MJ, Jula A, Leiviskä J, Palotie A, Salomaa V, Perola M, Ala-Korpela M, Peltonen L. Metabonomic, transcriptomic, and genomic variation of a population cohort. *Mol Syst Biol* 2010;**6**:441.
29. Kujala UM, Makinen VP, Heinonen I, Soininen P, Kangas AJ, Leskinen TH, Rahkila P, Würtz P, Kovanen V, Cheng S, Sipilä S, Hirvensalo M, Telama R, Tammelin T, Savolainen MJ, Pouta A, O'Reilly PF, Mäntyselkä P, Viikari J, Kähönen M, Lehtimäki T, Elliott P, Vanhala MJ, Raitakari OT, Järvelin MR, Kaprio J, Kainulainen H, Ala-Korpela M. Long-term leisure-time physical activity and serum metabolome. *Circulation* 2013;**127**:340–348.
30. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol* 1995;**57**:289–300.
31. Ritchie SC, Würtz P, Nath AP, Abraham G, Havulinna AS, Fearnley LG, Sarin AP, Kangas AJ, Soininen P, Aalto K, Seppälä I, Raitoharju E, Salmi M, Maksimow M, Männistö S, Kähönen M, Juonala M, Ripatti S, Lehtimäki T, Jalkanen S, Perola M, Raitakari O, Salomaa V, Ala-Korpela M, Kettunen J, Inouye M. The biomarker GlycA is associated with chronic inflammation and predicts long-term risk of severe infection. *Cell Syst* 2015;**1**:293–301.
32. Ebong IA, Goff DC, Rodriguez CJ, Chen H, Sibley CT, Bertoni AG. Association of lipids with incident heart failure among adults with and without diabetes mellitus: Multiethnic Study of Atherosclerosis. *Circ Heart Fail* 2013;**6**:371–378.
33. Nørrelund H, Wiggers H, Halbirk M, Frystyk J, Flyvbjerg A, Bøtker HE, Schmitz O, Jørgensen JO, Christiansen JS, Møller N. Abnormalities of whole body protein turnover, muscle metabolism and levels of metabolic hormones in patients with chronic heart failure. *J Intern Med* 2006;**260**:11–21.
34. Aquilani R, La Rovere MT, Febo O, Boschi F, Iadarola P, Corbellini D, Viglio S, Bongiorno AI, Pastoris O, Verri M. Preserved muscle protein metabolism in obese patients with chronic heart failure. *Int J Cardiol* 2012;**160**:102–108.
35. Izzo JL, Levy D, Black HR. Importance of systolic blood pressure in older Americans. *Hypertension* 2000;**35**:1021–1024.
36. Wannamethee SG, Welsh P, Whincup PH, Sawar N, Thomas MC, Gudnarsson V, Sattar N. High adiponectin and increased risk of cardiovascular disease and mortality in asymptomatic older men: does NT-proBNP help to explain this association? *Eur J Cardiovasc Prev Rehabil* 2011;**18**:65–71.
37. Packard CJ, Ford I, Robertson M, Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM, Gaw A, Hyland M, Jukema JW, Kamper AM, Macfarlane PW, Perry IJ, Stott DJ, Sweeney BJ, Twomey C, Westendorp RG; PROSPER Study Group. Plasma lipoproteins and apolipoproteins as predictors of cardiovascular risk and treatment benefit in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). *Circulation* 2005;**112**:3058–3065.
38. Kopple JD. Phenylalanine and tyrosine metabolism in chronic kidney failure. *J Nutr* 2007;**137**(6 Suppl. 1):1586S–1590S.
39. Schug ZT, Voorde JV, Gottlieb E. The metabolic fate of acetate in cancer. *Nat Rev Cancer* 2016;**16**:708–717.
40. Kang SM, Park JC, Shin MJ, Lee H, Oh J, Ryu DH, Hwang GS, Chung JH. ^{1}H nuclear magnetic resonance based metabolic urinary profiling of patients with ischemic heart failure. *Clin Biochem* 2011;**44**:293–299.
41. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GM, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2016;**18**:891–975.
42. Januzzi JL, van Kimmenade R, Lainchbury J, Bayes-Genis A, Ordonez-Llanos J, Santalo-Bel M, Pinto YM, Richards M. NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients. The International Collaborative of NT-proBNP Study. *Eur Heart J* 2006;**27**:330–337.
43. Goyal P, Almarzooq ZI, Horn EM, Karas MG, Sobol I, Swaminathan RV, Feldman DN, Minutello RM, Singh HS, Bergman GW, Wong SC, Kim LK. Characteristics of hospitalizations for heart failure with preserved ejection fraction. *Am J Med* 2016;**129**:635.e15–26.